PLANKTONIC ARMOURED PROPAGULES OF THE EXCAVATING SPONGE *ALECTONA* (PORIFERA: DEMOSPONGIAE) ARE LARVAE: EVIDENCE FROM *ALECTONA WALLICHII* AND *A. MESATLANTICA* SP. NOV.

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The armoured propagules of the excavating sponges *Alectona* and *Thoosa*, which are the only stage of reproduction of sponges consistently observed in full planktonic conditions, have been interpreted as asexual because of their unique morphology among sponge larvae. Evidence for their sexual origin is presented by new data for *Alectona wallichii* and a new species from the deep Atlantic, *A. mesatlantica* sp. nov. Stages in spermatogenesis and oogenesis are present at the same time as embryos in various stages of development, from early segmentation to advanced embryos possessing a discotriaene and style skeleton. Thick collagen strands surround the blastomeres from the beginning of segmentation. Collagen strands and spicules appear when the embryo cells are still undifferentiated. The larva is unique among Demospongiae in lacking surface flagella and having a larval skeleton, including spicules that are unknown in the adult. The name 'hoplitomella' is proposed for this special larva of alectonid sponges. The uniqueness of its sexual development and the tetraxonial nature of discotriaenes in larva of *Alectona* both indicate that these sponges do not belong to Hadromerida. A distinct family Thoosidae is supported for the genera *Thoosa*, *Alectona* and *Delectona*, although the family is considered presently as *incertae sedis* within Demospongiae. ☐ *Porifera, Demospongiae, Alectona wallichii, Alectona sp. nov., sexual reproduction, embryology, planktonic larva, excavating sponges, bioerosion.*

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The armoured propagules of the excavating clionid sponges *Alectona* Carter, 1879, and *Thoosa* Hancock, 1849, are intriguing from several points of view. These sponges produce unique reproductive bodies having a planktonic stage, and possessing a special skeleton of long protruding spicules and a cover of flattened spicules. Their sexual or asexual nature is unclear, although they have most often been considered as asexual products because of their unusual morphology, with the name 'armoured gemmules' generally applied to them.

These reproductive bodies have unique characteristics among Porifera. Firstly, the occurrence of a special spicule skeleton in propagules of sponges, otherwise not present in adults, is exceptional. Although spicules may be present in larvae, they are usually juvenile spicules resembling those of the adult (Bergquist & Sinclair, 1973; Simpson, 1984), or differing only in spination (Brien, 1967). The only other examples of a special skeleton in sponge reproductive bodies occur in Demospongiae, in the form of a microsclere skeleton in gemmules of the fresh-water Spongillidae (De Vos et al., 1991), and in Hexactinellida in the form of a stauractine skeleton in trichimella larva (Okada, 1928; Boury-Esnault & Vacelet, 1994). Moreover, these spicules in *Alectona* are discotriaenes with a clear tetraxonid origin. Tetraxon spicules are diagnostic for Spirophorida and Astrophorida, and are absent from Hadromerida in which *Alectona* is usually classified. *Alectona* and *Thoosa*, and by extension the family Clionidae in which they are usually included, have been considered intermediary between monaxinellids and tetractinellids (Topsent, 1891).

Secondly, the 'armoured gemmules' are the only example of organised sponge propagules consistently observed in the plankton. Dispersal stages of sponges are probably, but not certainly, present in a fully planktonic environment given that some species, especially Calcarea, are able to colonise experimental substrates several kilometres from the littoral in open sea conditions (Vacelet, 1981). Although several examples of 'pelagic plasmods' (Trégouboff, 1942), which were interpreted as fragments of various adult demosponges (Topsent, 1948), could actually have a role in sponge dispersal (Wulff, 1985, 1991), typical sponge larvae are almost never observed in the plankton, possibly because they are lecithotrophic with a short free life span normally spent near the bottom. The armoured propagules of *Alectona* were first described as a planktonic radiolarian (Karawaiew, 1896, 1897), and then identified as the larvae or gemmules described by Topsent (1903) in the living tissue of *Alectona* and *Thoosa* (Trégouboff, 1939, 1942). The area of Villefranche/mer in the Mediterranean, where *Alectona* and possibly *Thoosa* propagules have been observed, is especially rich in planktonic organisms and famous for the upwelling of deep-sea planktonic species (Trégouboff & Rose, 1957).

Planktonic armoured gemmules were the subject of controversy with acrimonious debate between the planktonologist Trégouboff and the spongologist Topsent. Trégouboff (1939, 1942, 1957) claimed that the skeleton, which is well-adapted to a pelagic existence, with protruding styles constituting flotation devices and a cover of discotriaenes protecting the mass of cells, develops during their planktonic life. The gemmules would mature in the plankton, the tetraxonial plates changing to monaxonial plates while amphiaster microscleres develop. The plates would then dissolved, while the styles progressively disappear, and the gemmules, having lost their special skeleton, supposedly fall to the bottom and metamorphose. Topsent (1941, 1948) maintained that the alleged stages in evolution were gemmules belonging to two different genera, i.e. *Alectona*, in which the plates are tetraxonial, and *Thoosa*, in which the plates are monaxonial. Topsent also questioned the usefulness of a heavy armour and special skeleton for a planktonic existence. The question has not been resolved, due to the rarity of observations that furthermore concern only part of the complete cycle. The evolution of the tetraxonial plates into monaxonial ones, and their progressive dissolution described by Trégouboff, appear rather unlikely; but the stages presumably belonging to *Thoosa*, according to Topsent, do not have the characteristic nodulose amphiasters of this genus.

The sexual or asexual nature of the 'armoured gemmules' also remained uncertain. They were first interpreted as sexual embryos (Topsent, 1903, 1904). However, their unusual morphology later inclined sponge biologists to the opinion that they were asexual (Topsent, 1920), and the name 'armoured gemmules' has been generally used, although they would be better referred to as 'armoured buds' (Simpson, 1984). In a modern cytological study Garrone (1974) concluded that their structure was clearly distinct from any type of sponge larvae or gemmules, but showed similarities with external buds.

I report here on some new data provided by specimens of a rare species from the Indo-Pacific, *Alectona wallichii* (Carter, 1874), recently rediscovered (Bavestrello et al., 1998), and here redescribed, and by a new deep-sea species, *A. mesatlantica* sp. nov., in which stages in reproduction demonstrate the sexual origin of these propagules.

MATERIALS AND METHODS

Specimens of *Alectona wallichii* from Hawaii were collected by the author while SCUBA diving in caves at Oahu, Hawaii. Rocky pieces of the walls were detached with a hammer and preserved in formalin.

Five specimens of the same species were collected excavating the skeleton of *Acropora sp.* on the outer reef flat of the 'Grand Récif', Tuléar, Madagascar. These were also preserved in formalin.

Specimens of *Alectona mesatlantica* sp. nov. were collected by means of the submersible 'Nautile' near St Peter & St Paul Rocks on the south Mid-Atlantic Ridge, at 2030m depth, during the 'SAINT PAUL' cruise in 1998.

Fragments of specimens were embedded in Araldite, either prior to or following desilicification in 5% hydrofluoric acid. Semi-thin sections were stained with toluidine blue. Polished sections including spicules and living tissue were obtained by sawing the embedded specimens with a low speed saw using a diamond wafering blade and wet-grounded with abrasive paper. The dissociated spicules were sputter-coated with gold-palladium, and observed under a Hitachi S570 scanning electron microscope (SEM). Observations with transmission electron microscopy were not possible due to the poor cytological preservation of specimens. Spicule dimensions are given as length/width.

Abbreviations: BMNH, The Natural History Museum, London; MNHN, Muséum National d'Histoire Naturelle, Paris; SME, Station Marine d'Endoume, Marseille.

SYSTEMATICS

Order **Incertae sedis**
Family **Thoosidae** Rosell & Uriz, 1997
Alectona Carter, 1879

Alectona wallichii (Carter, 1874)
(Figs 1-2, Table 1)

Gummina wallichii Carter, 1874: 252.
Corticium wallichii; Carter, 1879a: 353.
Alectona wallichii; Carter, 1879b: 496.

MATERIAL. HOLOTYPE: BMNH (not seen): Cape of Good Hope, South Africa, 146-182m depth. SPECIMENS: Unregistered: Shark Cove, on the NW coast of Oahu Island, Hawaii, 27.vi.1978, in a cave at 10m depth, coll. J. Vacelet. SME 1111(2), 1119(1-2), 1371(1), Grand Récif, Tuléar, Madagascar, 1971, outer reef flat, intertidal, coll. Brunel.

DESCRIPTION. *Morphology*. Specimens from Hawaii, collected from limestone rocks, excavate large, subspherical cavities, irregular in shape, 5-6mm diameter. Cavities are usually single, without the cateniform arrangement of chambers present in most clionids. The flesh is yellowish, with rather cartilaginous consistency, and contains numerous white ovoid or round embryos, 200-300µm diameter. Cavities communicate with the outside by tunnels (papillary canals) 1.5mm diameter, ending in papillary perforations of the same size. In tunnels, the sponge tissue is tougher than in chambers, and is organised around a central longitudinal cavity, 0.5mm diameter, with the large diactine spicules longitudinally arranged and with a strong concentration of amphiasters, similar to that described for *A. millari* Carter, 1879. The papillae, which are probably contracted, are grossly circular, without raised rim. They are closed at their outer end and no ostia or oscules are visible. Specimens from Madagascar were excavating on *Acropora* corals. The flesh is softer, whitish or yellowish, and the cavities are approximately 4-8mm diameter. Papillae were not observed.

Surface. In specimens from both localities, cavities show the characteristic pitted surface of excavating sponges (Rützler & Rieger, 1973), with subcircular depressions 50-80µm diameter (Fig. 1A, B). A few small perforations, 1-8µm diameter, irregularly dispersed among the pits, are probably due to boring Cyanobacteria or to fungi. The pits bear concentric lines and a secondary system of radiating lines (Fig. 1B).

Spicules (Figs 1C-H; 2A-C, E-F; Table 1). Smooth diactines, irregularly flexuous or bent at the centre, frequently with a swelling on the convex side of the central angle. Abnormal, monstrous forms are frequent. Ends are most often acerate, rounded in the thicker spicules. The large axial canal frequently makes an angle or a loop in the middle of the spicule, corresponding to the swelling. The smallest spicules often make a loop in the centre. A few spicules with two lateral actines have also been observed. Size: 280-490/10-28µm in Hawaii, 170-500/5-20µm in Tuléar.

Tuberculate diactines, of general shape similar to the smooth diactines, but wholly covered with button-like spines, 10-12.5µm diameter, regularly arranged along 12 longitudinal rows in which the tubercles alternate in adjoining rows. Shaft of the button smooth, enlarged surface covered by irregular tubercles. Spicules usually slightly bent; some flexuous or strongly bent in the centre. The large axial canal (0.6-1.2µm) frequently displays an angle or a loop in the middle of the spicule. A prominent axial filament is clearly visible on semithin sections after desilicification (Fig. 2D). A few tripod-like triactines are present. Size 380-500/40-50µm in Hawaii, 290-550/33-53µm in Tuléar.

Bumped or spinose diactines, are intermediary between smooth and tuberculate diactines. Size 350-560/22-30µm in Tuléar, absent in Hawaii.

TABLE 1: Spicule sizes (in µm) of the various known specimens of *Alectona wallichii*.

Spicules	Material					
	Hawaii	Tuléar 1111(2)	Tuléar 1119(1-2)	Tuléar 1371/1	Bavestrello et al. 1998	Carter 1874-79
Tuberculate diactines	380-500/40-50	290-310/33-35	320-390/30-38	470-550/50-53	678-713/70-93	805/62-125
Smooth diactines	280-490/10-28	290-350/11-20	170-410/5-20	430-500/18-20	468-631/21-38	-
Spinose diactines	-	350-370 /22-25	380/30	540-560/30 (rare)	-	-
Amphiasters	15-35	15-30	15-40	10-30	20-47/7-10	25/8.3
Discotriaenes	110-120	90-120	-	-	-	-
Styles	280-610/2-3	370-650/2-3	-	-	-	-

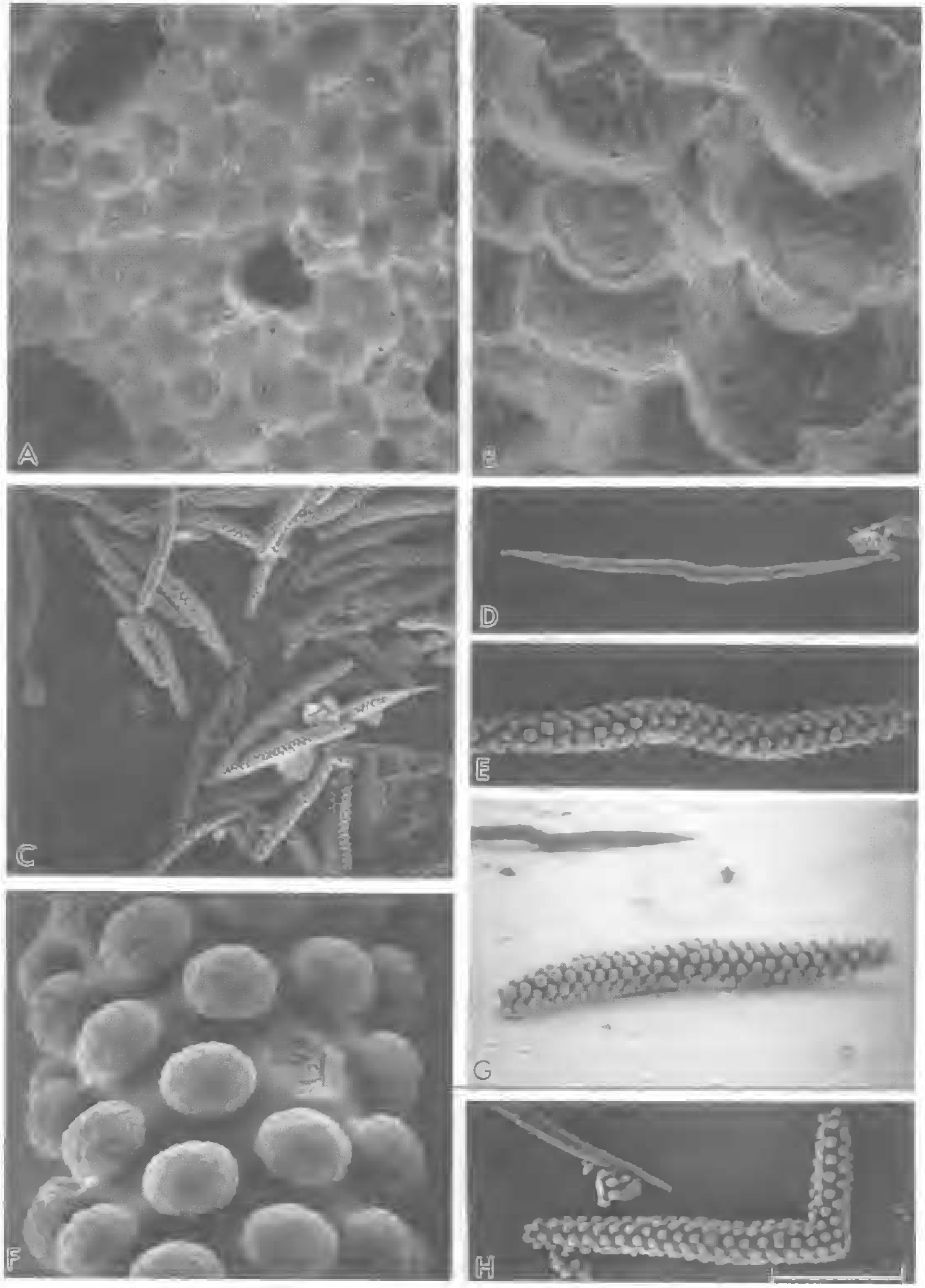


FIG. 1. *Alectona wallichii*. A-B, Pits on the surface of excavated scleractinian skeleton from Tuléar, showing a double system of concentric and radiating lines (Scale bar: A=152μm; B=51μm). C-H, Smooth and tuberculate diactines from Hawaiian specimens (Scale bar: C=374μm; D=126μm; E=126μm; F=19.6μm; G=136μm; H=142μm). Scale bar on bottom right.

Amphiasters, or more correctly sanidasteroid discorhabds, have short, blunt actines. Actines are generally equal and disposed in two whorls near the middle of the axis, but spicules with additional or unequal actines are frequent. Spicules are microspined, except in the central part of the axis between the two whorls of actines. A few small spicules, with an axis as short as 7.5µm, resemble the nodulose amphiasters found in the genus *Thoosa*. Size 15-35/2-2.5µm in Hawaii, 10-40µm in Tuléar.

Discotriaenes of the embryos have an irregularly circular outline, sometimes roughly triangular. Edge of the disc is slightly inwardly curved, with outer central part depressed. The inner surface bears a few small tubercles. The rhabdome is rarely accrate, most often with a blunt or inflated tip which may divide. An axial canal is clearly visible in the rhabdome, but cannot be traced in the disc. Size 110-120µm diameter with a rhabdome 20-30/8-10µm in Hawaii, 90-120µm in Tuléar (the latter observed in only a single specimen).

Styles of the embryos, are straight, slightly enlarged at some distance from the rounded end, with a large axial canal up to 1.2µm diameter. Two size categories are present in specimens containing advanced embryos: 280-360/1.5-3µm and 610-650/2-3µm, the former dominating in Hawaii and the latter in Tuléar.

The diactines are dispersed without order in the choanosome inside cavities. Walls of the papillary canals contain a greater concentration of longitudinally arranged tuberculate diactines (Fig. 2D). Amphiasters are dispersed throughout the whole tissue, although more numerous in papillary canals. The styles and discotriaenes are present only in embryos, the discotriaenes as an external layer, the styles as three fascicles made up of two spicules crossing in the centre of the embryo.

Living tissue. Living tissue of specimens from Hawaii is rather dense, with few canals (Fig. 3A-B). Choanocyte chambers are spherical, 14-20µm diameter. The mesohyl contains a large number of bacteria of the morphotypes usually found in bacteriosponges. The walls of the papillary canals are reinforced by dense, intertwined collagen fascicles containing elongated collencytes (Fig. 2D).

Specimens from Tuléar have the same general features as those from Hawaii, although their tissues are more poorly preserved. Choanocyte chambers are not visible. One specimen that has probably suffered from delayed fixation contains many rod-like bacteria, different in shape from the usual symbiotic bacteria.

Reproductive stages (Fig. 3A-F). Several stages of sexual reproduction are simultaneously present in the choanosome of most specimens. They are described here mostly from the Hawaiian specimens, which are better preserved and display more numerous stages.

Spermatogenesis occurs in spermatic follicles, which are spherical cavities, 25-40µm diameter, surrounded by a thin pinacocyte envelope. They contain densely stained spermatids or spermatocytes in various stages grouped as morulae at the centre of the follicle. In the most advanced stage observed (Fig. 3B), the spermatozoa are dispersed within the spermatic follicle. They have an elongated head measuring 2-3/0.5-0.6µm, and a long flagellum.

Oocytes are rare, with only two stages of their development observed. The youngest is a rounded cell lying in the mesohyl without a special collagen envelope, 20µm diameter, with the cytoplasm containing a few large inclusions, and a prominent, 6µm diameter nucleus containing a 2-3µm nucleolus. Another stage, which was observed only once in a thick section with poor definition, is 90µm diameter with an 18µm nucleus and a 4µm nucleolus. The cytoplasm contains large vitelline inclusions, and is surrounded by a follicular envelope of flattened cells and by a dense collagen envelope.

Embryos are spherical and uniform in size, approximately 200-320µm, regardless of their developmental stage (Fig. 3A). However, the most mature stages are slightly larger and elongated. Segmentation is total and equal. A four-cell stage has been observed, with apparently equal blastomeres 70-110µm diameter, filled up with heterogeneous vitelline inclusions 3-8µm diameter (Fig. 3C). Blastomeres divide without formation of a blastocoele. The early stages, up to the formation of the larval skeleton, are surrounded by a thin outer envelope made up of very thin, elongated cells, and by a dense inner layer, up to 10µm thick, made of collagen fascicles. Similar fascicles also individually wrap the blastomeres (Fig. 3C, D). This collagen development, which is highly unusual in sponge embryos, is maintained during the following stages. The most advanced embryos observed are made up of cells, 10-15µm size with inclusions 5µm diameter, which are widely dispersed in a loose collagen matrix also containing dispersed

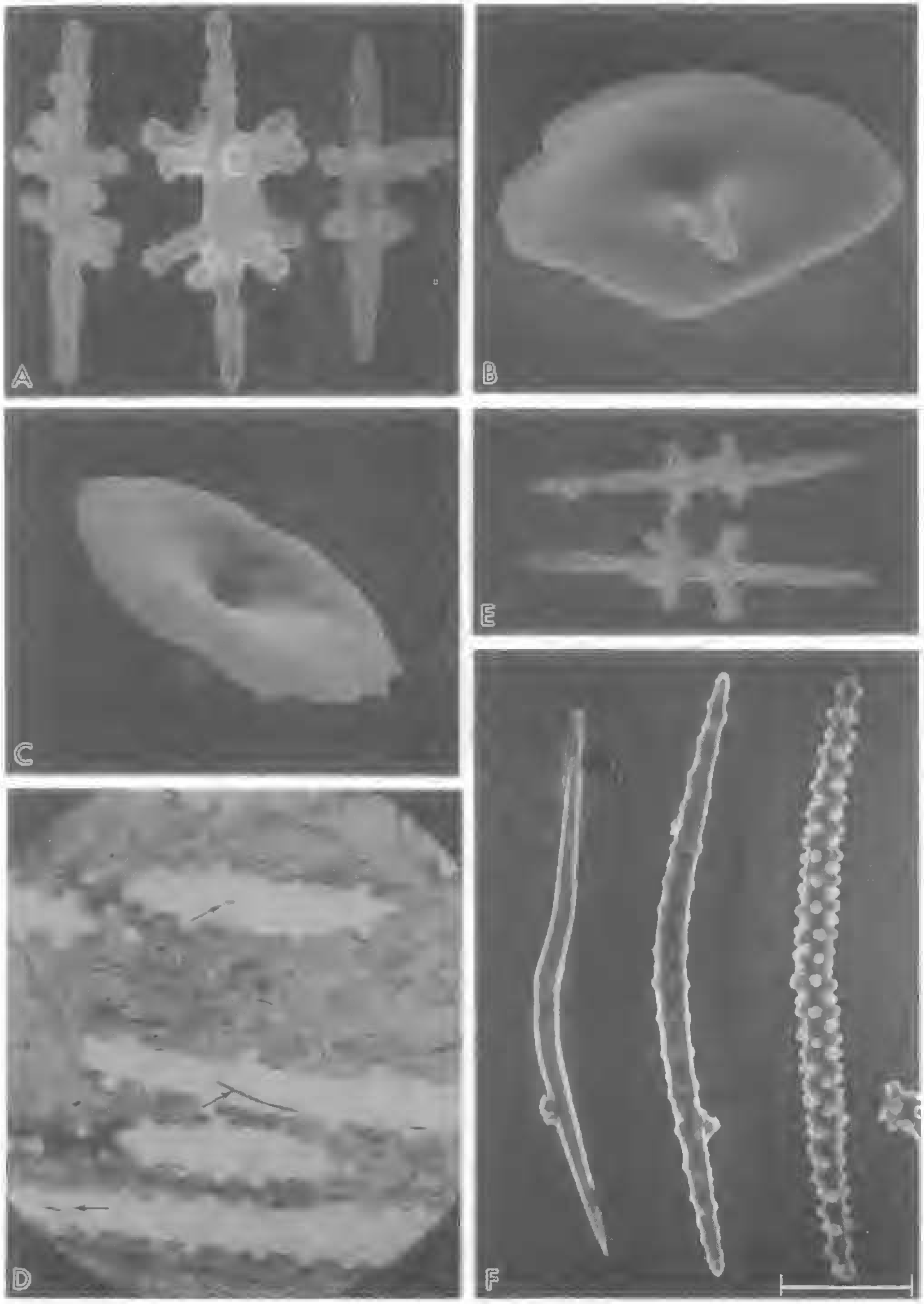


FIG. 2. *Alectona wallichii*. A-C, Spicules from Hawaiian specimens. A, Amphiasters. B, C, Discotriaenes (Scale bar: A=11μm, 6.2μm, 12.5μm from left to right; B=40μm; C=44μm). D, Semi-thin section through the papilla after desilicification, showing collagen strands, collencytes and axial filament (arrows) in the spicule ghosts (Scale bar=52μm). E-F, Spicules from Tuléar specimens. E, Amphiasters. F, Smooth, spinose and tuberculate diactines (Scale bar: E=15.8μm; F=86μm, 72μm, 98μm from left to right). Scale bar on bottom right.

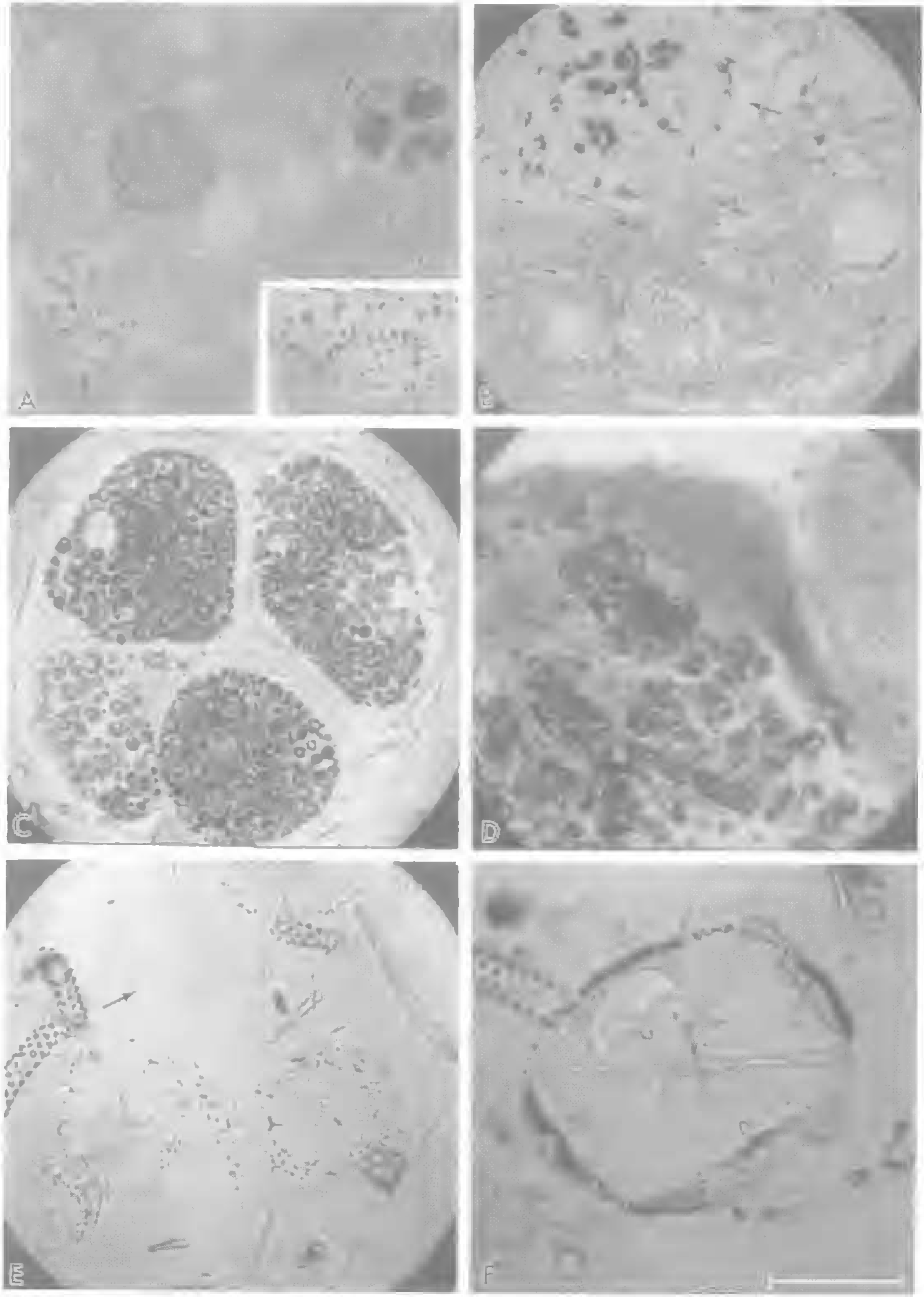
symbiotic bacteria. A group of elongated cells perpendicular to the embryo surface is observed near one pole of the mature embryo on a slightly prominent button (Fig. 3A). Most cells still appear to be undifferentiated. Contrary to the free-living larvae of *Alectona millari* (Garrone, 1974), these embryos possess neither choanocyte chambers nor spherulous cells.

The larval skeleton appears when the blastomeres have grown to approximately 20µm diameter and are still apparently undifferentiated. The three spicule categories appear simultaneously, first as very thin (1-1.5µm) styles and incomplete discotriaenes, in which the smaller disc observed attained only 20µm diameter (Fig. 3E). Amphiasters are recognisably the same as in later stages, but are less numerous than those found in the most advanced embryos. In advanced stages (Fig. 3F), the embryo is surrounded by a single layer of discotriaenes in which the shaft is inwardly directed. The cover is first discontinuous, then becomes continuous with a total number of approximately 12 discotriaenes. Styles, consistently six in number in each embryo, are disposed in three fascicles made up of two parallel spicules, which cross approximately at right angles to each other near the centre of the embryo. Amphiasters are mostly located near the discotriaene cover, although a few are dispersed within the internal tissue. The most advanced embryos observed had relatively short styles, which will probably eventually elongate and finally rearrange at one pole, as observed in *Thoosa* (Topsent, 1904), before being shed through the canals at later developmental stages.

Specimens from Tuléar also have several reproductive stages. Rare spermatic follicles were observed, with the same features as those seen in Hawaii. Embryos were mostly in early stages of development, with blastomeres 30-50µm diameter, and without spicules. They differ from those of Hawaiian samples by a greater development of the collagen envelope, which is up to 25µm thick and made up of intertwined collagen fascicles (Fig. 3D). The fascicles in between the blastomeres are also larger (approximately 10µm) than in Hawaiian material. Mature stages retaining a larval skeleton were not observed in sections, although they were present in one specimen, as indicated by the presence of larval spicules on dissociated spicule preparations (Table 1).

REMARKS. As summarised in Pang (1973, 1977), *Alectona wallichii* was originally described in the genus *Gummina* by Carter (1874), from isolated acanthodiactines collected from the Cape of Good Hope at 146-182m depth. These remarkable spicules were previously described by Bowerbank (1864), also as isolated spicules washed off corals. Carter (1879a) later succeeded in finding the whole sponge and its complete spiculation from the same skeletons of *Stylaster* studied by Bowerbank, and subsequently transferred the species to *Corticium*. He discussed its possible excavating habitus and gave three probable localities inferred from the known occurrences of the highly diagnostic spicules: the 'South Sea', Cape of Good Hope and the Seychelles. Carter (1879b) later transferred the species to *Alectona*, where it has been considered either as the type-species of the genus *Alectona* or as a synonym of *A. millari* (Laubenfels, 1936), although no additional material was known until the species was rediscovered in the NW Pacific by Bavestrello et al (1998). It is likely that *A. wallichii* was already present in the Early Miocene, as suggested by tuberculate spicules found in sediments from the W Atlantic figured by Wiedenmayer (1994).

Although some individual variations were observed between the various specimens from the Indian and Pacific Oceans (Table 1), the species appears to be well-characterised by its tuberculate diactines. These diactines are generally smaller in material from Tuléar (with the exception of specimen 1371/1, which has the largest megascleres). In all specimens, however, megascleres were smaller than in those recorded in the type-specimens by Carter (1874, 1879a) and in material from the NW Pacific (Bavestrello et al., 1998). The most important difference between material from Hawaii and Tuléar, however, is the presence of spinose or bumped diactines, which are intermediary stages between tuberculate and smooth diactines, in Tuléar specimens. The presence of these intermediary spicules in Tuléar specimens, which have few or no advanced embryos, could indicate that diactine production, first appearing as smooth spicules, is reduced in actively reproducing specimens. The discotriaenes and styles are present only in specimens containing advanced embryos.


A
B
C
D
E
F

Alectona mesatlantica sp. nov.
(Fig. 4)

MATERIAL. HOLOTYPE: MNHN D JV 63: Saint Peter & Saint Paul Rocks, mid-Atlantic Ridge, 00°94'N, 25°29'W, 2030m depth, 6.i.1998, coll. 'Nautile' submersible, R.V. 'Saint Paul' (sample SP13-16).

ETYMOLOGY. From *mes*, Greek, middle, and Atlantic, pertaining to the type locality on the Mid-Atlantic Ridge.

HABITAT. In deep water (2030m depth), near to Saint Peter & Saint Paul Rocks in the Equatorial Atlantic; excavating large cavities in calcareous rock of probable organic origin, encrusted by ferromanganese oxyhydroxides.

DESCRIPTION. *Morphology and living tissue* (Fig. 4A). The sponge is a fleshy mass growing in tunnels or in subspherical cavities, up to 5cm maximum dimension, single but irregular in shape. Communication with the outside is by a few papillary canals, 2-3mm diameter, ending in papillae of the same size with a single aperture, 1mm diameter. Colour is white in alcohol. The tissue is rubbery, compact with few aquiferous canals 2-3mm diameter. The mesohyl is typical of bacteriosponges, containing numerous, densely packed symbiotic bacteria. There are no visible cells with inclusions. Choanocyte chambers were poorly preserved, 22-30μm diameter. Aquiferous canals are lined by elongated cells aligned parallel to the canals, collagen fascicles and diactines.

The skeleton, composed of acanthodiactines and amphiasters, is developed only along the canals and near the border of the cavities. It is reduced and nearly absent in the choanosome. Styles and discotriaenes are found only in spiculate embryos.

Cavities display the characteristic pitted surface of excavating sponges, with subcircular pits 25-42μm diameter (Fig. 4B). Pits have an irregular surface, with concentric lines and secondary small holes. This structure is visible on all the components of the limestone construction, indicating that it is not related to the microstructure of the bored substrate.

Spicules (Fig. 4C-F). Acanthodiactines are of very irregular shape, usually with a swelling and bent in the middle, sometimes with a more-or-less developed third ray, or one of the two rays absent. Axial canal are large, frequently vesiculated or making a loop near the middle of the spicule or near the occasional branching of a third ray. Spines are acerate, short, irregularly distributed. Size: 410-530/21-28μm without spines.

Amphiasters, with a relatively thick axis, are microspined except near the centre of the axis, with rounded actines predominantly central, sometimes disposed in two irregular whorls especially in the shorter spicules. Size: 20-60/3-7μm. Amphiasters of the spiculate embryos have a thinner axis than those of the body.

Styles of embryos are very thin, flexuous, slightly enlarged at some distance from the rounded end, mostly broken on the slides. Size: up to 1125/4-5μm.

Discotriaenes of embryos have a short rhabd (20-40μm), frequently dichotomous or with lateral expansions. Disc is 130-150μm diameter, circular or slightly triangular, with the three branches of the axial canal clearly visible. Incomplete spicules, including a 'triaene' with free clads whose surface is irregular, were observed in embryos in the early stage of spiculogenesis.

A few large, irregular siliceous plates, probably of foreign origin, were observed in dissociated spicule preparations. Their position in the sponge tissue is unknown.

Acanthodiactines and asters are very rare or absent in the choanosome. They are more abundant near the walls of the excavation, and in the lining of the choanosomal and papillary

FIG. 3. *Alectona wallichii*. Stages in reproduction in specimens from Hawaii (except D, from Tuléar). A, Semi-thin section of desilicified tissue, general view of choanosome with young embryos at diverse stages in segmentation, and an embryo with ghost of discotriaene cover (arrow) enlarged in the inset (Scale bar=218μm, inset=142μm). B, Semi-thin section of desilicified tissue, nearly mature spermatic follicle and advanced embryo with ghost of discotriaene cover (arrows) (Scale bar=56μm). C, Semi-thin section of desilicified tissue, early stage in segmentation with collagen strands between the blastomeres (Scale bar=56μm). D, Semi-thin section of desilicified tissue, specimen from Tuléar, embryo with thick collagen strands (Scale bar=38μm). E, Non-desilicified thick section, early stage in segmentation (arrow) and three embryos with developing discotriaenes and styles; a fully developed discotriaene of a mature embryo on bottom left (Scale bar=200μm). F, Non-desilicified thick section, advanced embryo with discotriaene cover and three fascicles of two styles (Scale bar=132μm). Scale bar on bottom right.

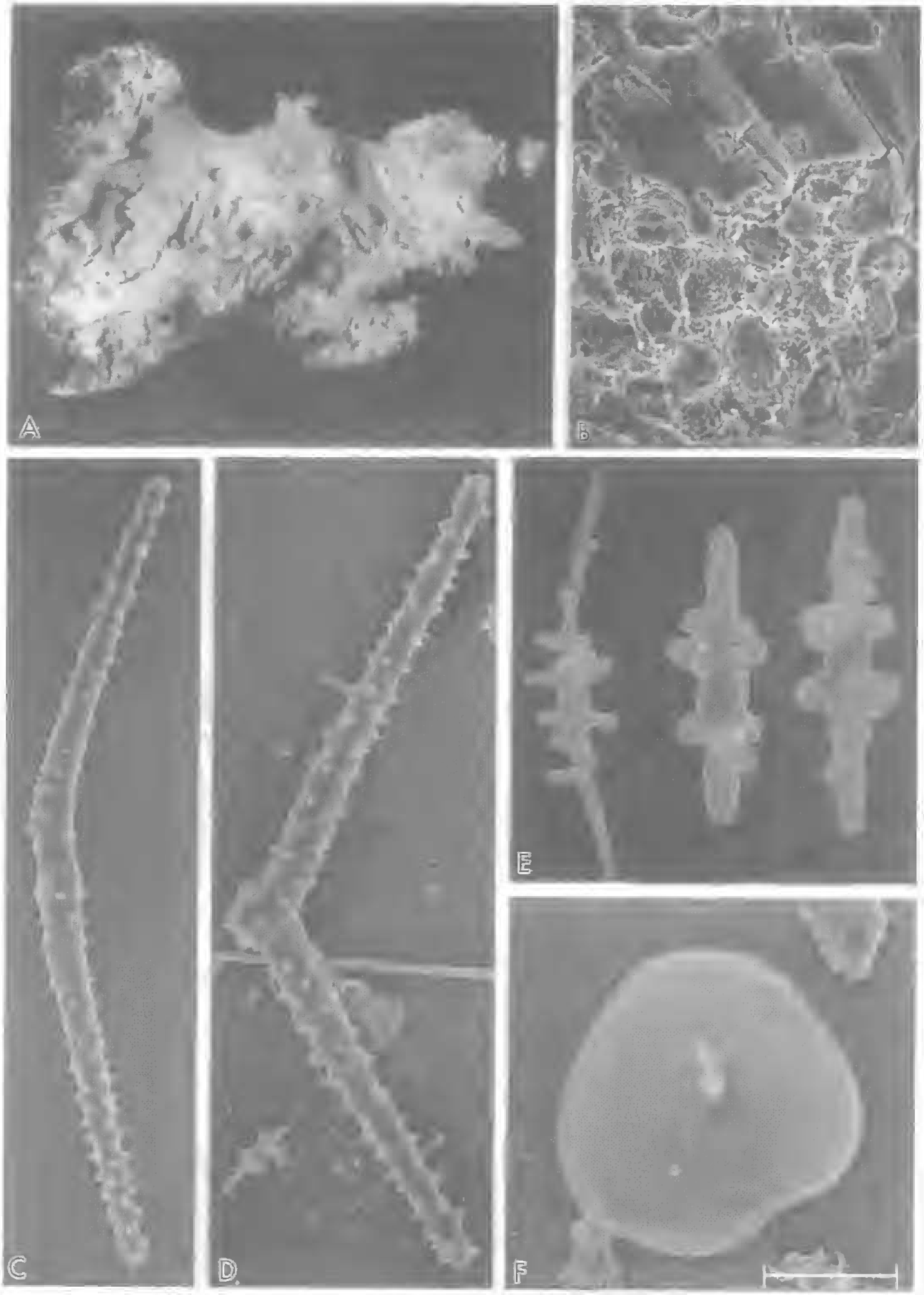


FIG. 4. *Alectona mesatlantica* sp. nov. A, Holotype (Scale bar=15.6mm). B, Pits on the excavated surface (Scale bar=64µm). C-D, Acanthodiactine (Scale bar: C=84µm; D=78µm). E., Amphiasters (Scale bar=20µm, 17.2µm, 17.2µm from left to right). F, Discotriaene (Scale bar=72µm). Scale bar on bottom right.

canals. Styles and discotriaenes are found only in advanced embryos, with the same arrangement as in *A. wallichii*.

Reproductive stages. The tissue contains numerous spermatic follicles, approximately 25µm diameter, at various stages of development ranging from a central mass of dense, 4µm diameter cells, to nearly mature spermatozoa with an elongated head 3/1µm.

The single stage of oogenesis observed was an oocyte with a poorly preserved cytoplasm, 40/20µm, with a nucleolate nucleus. Numerous ovoid embryos are present in the tissue. They display several developmental stages with a uniform size, 200-230/150µm. The earliest stage observed is a four-cell blastula with equal blastomeres, 100µm diameter, with numerous vitelline inclusions, 2-8µm diameter. These blastulae are surrounded by a thin envelope. Collagen strands, up to 35µm thick, are located between this envelope and the blastomeres. Thinner strands also wrap the blastomeres individually. Spicules first appear in embryos with an undetermined number of blastomeres, whose size is reduced to approximately 20µm, with inclusions smaller than 5µm. In these embryos the amphiasters are thinner than in the adult tissue, and the disc of the discotriaenes is incomplete, with the four axial filaments clearly visible. In the most advanced stages containing a complete larval skeleton, the blastomeres are smaller (approximately 10µm diameter), but apparently still undifferentiated and contain heterogeneous inclusions up to 4µm diameter. Intercellular bacteria are numerous in the embryo tissue. Discotriaenes are disposed as an outer cover over the embryo, and are surrounded by a thin tissue strand. Long styles are grouped either in a fascicle protruding from one pole into the maternal tissue, or in three perpendicular fascicles of two spicules.

REMARKS. The new species is characterised by the large size of its acanthodiactines, amphiasters with rounded spines, the unusually large cavities it excavates, the small size and peculiar aspect of the pits, and the distribution of megascleres which are virtually absent from the choanosome. The most closely related species appears to be the common North Atlantic-Mediterranean *A. millari* Carter, 1879, which bores smaller cavities with pits up to 100µm diameter, thus considerably larger than those of *A. mesatlantica*, which are smaller than 50µm, and whose diactines are smaller (Bavestrello et al., 1998) - although a Mediterranean specimen of *A. millari* has been recorded with tuberculate diactines 240-460/27-54µm (Pulitzer-Finali, 1983).

This is the deepest record for the genus. *Alectona millari*, previously considered to be a deep-sea species (Topsent, 1900), has now been found at 0.5m depth and is not recorded deeper than 1190m. The present species is also the deepest record for an excavating sponge after *Cliona levispira*, recorded at a depth of 2165m (Topsent, 1928b).

DISCUSSION

SEXUAL ORIGIN OF THE EMBRYOS. Observations made here have some similarities to those reported for *Thoosa armata* by Topsent (1904). Both these data demonstrate, in my opinion, that the 'armoured gemmules' are clearly larvae of sexual origin. However, this point is equivocal and requires further discussion because these are peculiar larvae, with characteristics generally found not in larvae, but rather in asexual gemmules or buds, and also because an asexual origin for 'larvae' of incubating demosponges has been alleged by several authors (Wilson, 1894; Sivaramakrishnan, 1951; Bergquist et al., 1970) – although their interpretation has been challenged and has never really gained general acceptance (Bergquist, 1978; Simpson, 1984). According to these authors, 'asexual larvae' may be found together with stages of sexual reproduction. The occurrence of sexual products (oocytes, spermatic follicles and indisputable embryos) simultaneously with the armoured bodies in *Alectona* is thus not full evidence that they are of sexual origin. However, considering the observed sequence it appears very unlikely that the armoured bodies would not be derived from the early segmentation stages. It may also be argued that oocytes are fewer in number than embryos, but this is a frequent phenomenon in incubating sponges. The presence of collagen fascicles between the blastomeres is highly unusual in sponge early embryos, as discussed below, but this is not a convincing argument for an asexual origin because collagen fascicles are also unknown between gemmular archaeocytes. Finally, the present data, particularly the observed sequence in development, leave no doubt as to the sexual origin of these reproductive bodies.

These embryos, however, display a number of unique peculiarities among poriferan larvae. 1)

They possess a larval spicule skeleton which is absent from the adult, a feature known only so far in the hexactinellid trichimella larva. 2) They are devoid of flagellae. 3) They have a strong development of collagen structures appearing at the end of the oocyte development and during the first segmentation stages. 4) They presumably have a long planktonic life with special flotation devices.

Such larvae clearly cannot be included in any other types of embryonic development in Porifera (Borojevic, 1970; Simpson, 1984; Fell, 1989), and the new term 'hoplitomella' (from *hoplites*, Greek, armoured, with heavy armour, and the suffix *mella*, used for some other types of sponge larvae), is proposed here. The hoplitomella is presently known only from the two genera *Alectona* and *Thoosa*.

MODE OF DEVELOPMENT. The early embryonic development of the hoplitomella larva is normal in that the first cleavages are equal and give rise to a solid stereoblastula, as in most incubating demosponges. However, a strong development of collagen occurs, both around the mature oocyte and in the embryo. The origin of collagen fascicles is not clear, and an ultrastructural study is needed to resolve this point. However, it is speculated that collagen fascicles surrounding the oocyte could be made either by the maternal sponge tissue, or by the oocyte itself. A synthesis of collagen fibrils by oocytes, which has been very rarely reported in animals (Wischnitzer, 1966), has been described in some oviparous demosponges (Gallissian & Vacelet, 1976; Watanabe & Masuda, 1990). The collagen envelope in early stages of the embryo may be derived from the oocyte envelope, as in the direct development of *Tetilla*, in which radiating fiber bundles of the egg surface are enclosed in the perivitelline space after fertilisation (Watanabe, 1978). This hypothesis, however, would neither explain the greater thickness of the embryo envelope, nor the presence of thick collagen fascicles between the blastomeres. It appears that in hoplitomella development the blastomeres are able to secrete thick collagen strands, even during the first cleavages. Although collagen fibrils are present in mature sponge larvae, they appear at a relatively late stage when most cell categories are already differentiated and they never form such thick, intertwined fascicles.

Another peculiarity is the early synthesis of the larval spicule skeleton, which also occurs at a time when most of the blastomeres are apparently still undifferentiated. The precocious appearance of siliceous spicules has been described in some demosponge embryos (Brien & Meewis, 1938; Fell, 1969; Simpson, 1984), but this occurs after differentiation of most cell types. It appears that the cells at the surface of the embryo, which in other sponges differentiate into a flagellated layer, differentiate here into discrete sclerocytes. As for precocious collagen synthesis, an ultrastructural study of the phenomenon would be of the greatest interest.

Differentiation of macromeres and micromeres, which is the subsequent step in embryonic development in other poriferans having a stereoblastula, resulting in differentiation of flagellated cells, appears to be skipped in this scheme. There is no early separation of a flagellated cell lineage. This separation probably occurs in a late developmental stage when the first choanocyte chambers differentiate in the free larvae, as described in *A. millari* (Garrone, 1974). Embryos observed here, as well as the free larval stages found in the plankton (Trégouboff, 1942; Garrone, 1974), are devoid of surface flagellae. This is again unique amongst the Porifera, where larvae are always more-or-less mobile due to the presence of flagellated cells, even in the creeping larvae of *Polymastia* (Borojevic, 1967). Flagellated cells are absent only in the development of *Tetilla*, whose zygotes develop directly without any larval stage (Watanabe, 1978). It remains to be confirmed, however, whether the mature larva is actually devoid of flagellae. The elongated cells grouped on a small protuberance in a pole of the embryo of *A. wallichii* (Fig. 3A) vaguely suggest a future small tuft of flagellae, although it has not yet been reported in free larva of *A. millari* (Trégouboff, 1942; Garrone, 1974).

After long debate, it has finally been convincingly demonstrated, both in Calcarea and in Demospongiae, that at metamorphosis the choanocytes of the young sponge may derive from larval flagellated cells (Amano & Hori, 1993, 1996, 1998). There is, however, evidence from other species for an absence of such a 'reversal of layers' (Misevic et al., 1990). The present observations, reporting the absence of flagellated cells in alectonid larvae, suggest that a direct lineage from larval flagellated cells to choanocytes cannot be retained in this development.

The absence of surface flagella, and consequently of swimming behaviour, is surprising in these larvae which are the only sponge elements regularly found in full planktonic conditions. It might have been supposed that such larvae with a presumed relatively long pelagic life would have a well developed swimming apparatus. Although the swimming devices of sponge larvae do not allow significant movement, they could play an important role by changing the vertical position in the water column or for final microhabitat selection before settlement. Instead, the hoplitomella appears to rely on flotation devices produced by its long protruding styles and, at least at the stages which have been observed, is completely at the mercy of the currents. The possibility that a swimming apparatus will develop at the end of the pelagic life, assuring refinement in the choice of substratum as in other poriferan larvae, is unknown but cannot be excluded at present. The absence of surface flagella would exclude photonegative behaviour, which is nevertheless likely as most species of *Alectona* and *Thoosa* are sciaphilous. Whether or not this peculiar mode of larval life has some effect on their dispersal ability and geographic distribution remains to be investigated.

GALLERY SIZE AND PIT ORNAMENTATION. The shape, size, and organisation of the camerate borings of excavating sponges have been tentatively used in systematics of clionids and for identification of species of the ichnogenus *Entobia* Bronn, 1837, with an evident interest as indicators of palaeoenvironments (Bromley, 1970, 1978; Bromley & D'Alessandro, 1984; Pleydell & Jones, 1988; Bromley et al., 1990; Edinger & Risk, 1996). The chambers of the two species of *Alectona* appears, from present data, to be larger than in most clionids, especially those of *A. mesatlantica* which range up to 5cm diameter. Another difference is that these large chambers are simple, apparently without any growth stage in which they would be camerate or catenate as in clionids.

The possible use of the fine features of the pits for a correlation between extant species and ichnospecies of excavating sponges has yet to be explored. The genus *Alectona* appears to have a special ornamentation of the pits, with a double system of concentric and radiating lines whatever the nature of the substratum. This has been observed in the skeleton of the calcified calcareous sponge *Petrobiona massiliana* bored by *A. millari* (Omnes, 1991), and is confirmed here in *A. wallichii*. The small size and the peculiar ornamentation of pits in *A. mesatlantica*, whose spiculation is not very different from that of *A. millari*, appears as an interesting additional taxonomic feature. This demonstrates that characterisation of both Recent species and the ichnospecies of genus *Entobia* using microsculpture of the boring walls is worth considering.

SYSTEMATIC POSITION OF *ALECTONA*. The systematic position of *Alectona* and related genera (*Thoosa* and *Delectona* Laubenfels, 1936, possibly with *Thooce* Laubenfels, 1936 and *Annandalia* Topsent 1928, which are probable synonyms of *Thoosa*) is puzzling. These genera are generally classified within Clionidae (Hadromerida) based on their excavating habit – a character of doubtful value given that it occurs within other orders of Demospongiae – and the presence of amphiasters that resemble those of *Cliothosa* Topsent, 1905, which undoubtedly belongs to the Clionidae (Topsent, 1928a; Rosell & Uriz, 1997). This classification has been questioned by several authors. Topsent (1891, 1900, 1928a) considered that the spinose diactines of *A. millari* were giant microscleres derived from oxyasters rather than from megascleres, and suggested that clionids, and in particular *Alectona*, were intermediary between Hadromerida and Tetractinellida, whereas Alander (1942) firmly classified *Alectona* and *Thoosa* in Tetractinellida. De Laubenfels (1936) suggested that Clionidae could be divided into two groups, possibly of the subfamily rank, i.e. *Cliona* and related genera, and *Thoosa* and related genera.

More recently, recognition of Thoosidae as a distinct family of Hadromerida has been proposed, first as Alectonidae (Rosell, 1996), then as Thoosidae (Rosell & Uriz, 1997). This distinction appears fully justified from my results. The presence of discotriaenes in larvae, to which may be added now the unique features of the sexual development, precludes their classification in Clionidae. These spicules are undoubtedly of tetraxonial origin, and thus also preclude the classification of *Alectona* in Hadromerida. Alternatively, they may be considered as an 'ancient adult character' (Jägersten, 1972), still present during embryonic development but disappearing in the adult during ontogeny. I will leave for the moment the family Thoosidae *incertae sedis*. Based on presently accepted criteria, its classification in Tetractinellida would rest only on the presence of discotriaenes in larvae of *Alectona*, whereas the tetractinellid character is unclear in *Thoosa* and *Delectona*. Furthermore, the larval development is markedly different from both Tetractinellida and Hadromerida, which are oviparous. Possibly a new order will be needed for these sponges when additional data becomes available on their full life cycle, cytology and molecular sequences.

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